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C07C 229/26
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(54) Use of copper complexes of alpha-amino acids that contain terminal amino groups as nutritional supplements

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22.08.89 US 396685
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- (68) References cited:  
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CHEMICAL ABSTRACTS, vol. 101, 1984, page  
526, abstract no. 160386n, Columbus,

Ohio, US; B.N. MIARA et al.: "Infrared study  
of some amino acids and their copper com-  
plexes", & INDIAN J. PURE APPL. PHYS. 1984,  
22(7), 430-2

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Rank Xerox (UK) Business Services  
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## Description

BACKGROUND OF THE INVENTION

5 The important physiological role of copper in vertebrates was first demonstrated in 1928. Since that time, copper was found to be a key component of several enzymes. Most of these copper-containing enzymes are known to catalyze physiologically important reactions. Dietary deficiency of copper in vertebrates results in several pathological disorders.

One early manifestation of copper deficiency is spontaneous fracture of bones in sheep and cattle that  
10 graze in copper-deficient pastures. Experimentally induced copper deficiency resulted in bone abnormalities in rabbits, pigs, chickens and dogs. The bone defect appears to reside in the organic matrix and not in the mineral make-up of the bone.

Copper deficiency results in the failure of pigmentation of hair and wool in numerous species including black-wooled sheep in which bands of pigmented and unpigmented wool can be correlated with copper  
15 supplementation. Feathers of turkey poults fed copper-deficient diets also show lack of pigmentation.

Copper deficiency can cause cardiac lesion in cattle which may even result in sudden death. Further, chicks and pigs fed copper-deficient diets may die suddenly from massive internal hemorrhage due to structural defects in major arteries.

Subnormal levels of copper in the forage has resulted in neonatal ataxia or swayback in lambs. Copper-  
20 deficient pigs do not absorb iron at a normal rate. It appears that copper is also essential for the release of iron from the intestinal mucosa and iron storage tissues. In general, copper deficiencies cause anemia in all the species that have been studied so far.

Only a fraction of the copper ingested in diet is ultimately utilized in the formation of copper-proteins. The form of the copper ingested influences the degree of its utilization as measured by ceruloplasmin  
25 (copper-protein enzyme) activity. The presence of some amino acids have been known to cause an increase in the absorption and utilization of copper. (Kirchgessner and Girassmann, Z. Tierphysiol. Tierernahrung Futtermittelk. 26:3 (1970; Girassmann et al., Z. Tierphysiol. Tierernahrung Futtermittelk. 28:28 (1971); Schwarz et al., ibid. 31:98 (1973).)

It can, therefore, be seen that proper dietary balance of highly bioavailable copper is important for  
30 animals, including swine and poultry. In recent times, it has been reported, particularly for swine and poultry, that they may have copper deficiencies because of the nature of their food rations. Put another way, it has been found that from time to time, mycotoxins which are present in food rations, particularly corn, have a tendency to tie up the available copper in a form which makes it non-bioavailable. Thus, the net result is that the feed for the swine and the poultry containing mycotoxins may in some way bind the  
35 available copper in the food to make it non-bioavailable. The net result is that even though one feeds to the animals much higher levels of copper than the NRC (National Research Council) recommended daily allowances, the animal does not get anywhere near its adequate NRC requirement.

An additional problem is caused by feeding high levels of copper which are simply thereafter excreted without uptake. The copper in the excrement is returned to the soil, significantly raising the copper level.  
40 This may cause undesired environmental polluting problems.

It therefore can be seen that adequate dietary levels of highly bioavailable copper are necessary for development of livestock, including cattle, swine and poultry. Moreover, there is a need to present copper in a highly bioavailable form which assures that the animal will have a high level of uptake of the copper without excreting it to cause potential environmental pollution.

45 It is an especially preferred advantage to make the complexes of the present invention from essential amino acids. This allows not only for increased bioavailability of the copper, but also it allows the animal a proper nutritional amount of an essential amino acid necessary for proper growth. For copper, it is especially difficult to form solid form complexes of copper and alpha-amino acids. For example, if one attempts to form such a complex with methionine in accord with the general procedures outlined in U.S.  
50 Patent 3,941,818 issued March 2, 1976 and U.S. Patent 4,021,569 issued May 3, 1977, the result is an oxidation-reduction reaction between the methionine and the copper, resulting in an unuseable reaction product. It has been discovered that when copper complexes of alpha-amino acids having a terminal amine group are employed, the internal redox reaction does not occur. In this respect, reference may be made to CHEMICAL ABSTRACTS, vol. 101, 1984, p. 526, n° 160386n; vol. 81, 1974, p.430, n° 84064g; vol. 90,  
55 1979, p.413, n° 70890x; vol. 84, 1976, p. 630, n° 114577h; vol. 101, 1984, p. 559, n° 6397s; vol. 86, 1977, p.673, n° 99387s; JOURNAL of the AMERICAN CHEMICAL SOCIETY, vol. 92, n° 5, 1970, p.1284-1289; and JOURNAL of MEDICINAL CHEMISTRY, vol. 19, n° 1, 1976, p.135-148.

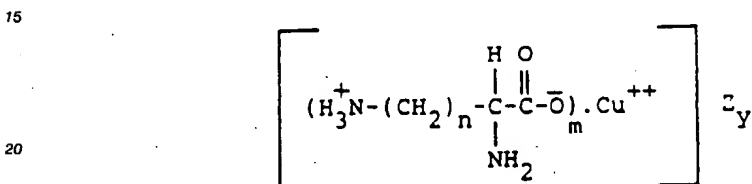
It is therefore an objective of the present invention to provide a method according to claims 1 to 6 of supplementing an animal's feed with an appropriate additive to assure adequate dietary requirements of copper for growth and health, using as said additive complex salts of copper and certain alpha-amino acids containing a terminal amine moiety, in which the copper is in a form that can be readily absorbed after ingestion by livestock, particularly swine, poultry and cattle.

A further objective of the present invention is to provide an animal's feed according to claim 7 comprising said additive.

An even further objective of the present invention is the use according to claim 8 of said complex salts for the manufacture of feed additives in animal nutrition.

In a more specific aspect of the present invention, said complex salts are 1:1 ratio complexes wherein the ratio of copper to the alpha-amino acid is 1:1 or 1:2 ratio complexes wherein the ratio of copper to the alpha-amino acid is 1:2.

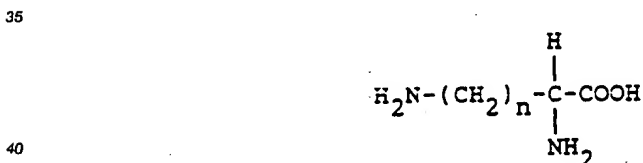
According to the present invention, the complex salts have the following general formula :



wherein "n" is from 1 to 5, "m" is 1 or 2, "Z" is an anion and "y" is the number required to electrostatically balance the set. Because of a complex formed between the copper and the alpha-amino acid, it is believed these compounds are in a form that is readily absorbed by domestic livestock, including cattle, swine, and poultry. These complexes thus function as a readily available source of copper for dietary supplementation while simultaneously providing essential alpha-amino acids for normal growth and health.

### DETAILED DESCRIPTION OF THE INVENTION

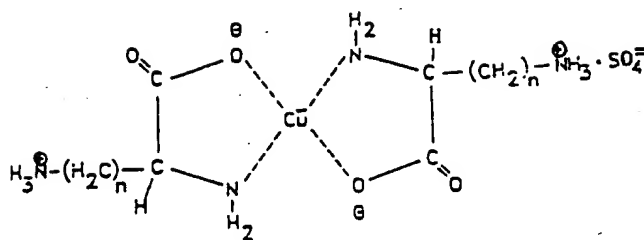
The compounds of the present invention can be described as copper complex salts between the copper cation and certain alpha-amino acids with a terminal amine moiety of the general formula:



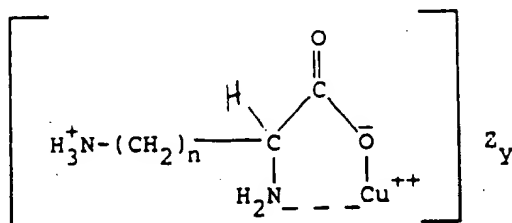
wherein "n" equals 1-5. The preferred acid is when "n" equals 4, lysine. However, "n" should not increase substantially beyond 5 because compounds for "n" greater than 5 are not readily available and not commercially feasible.

It is also important to note that the compounds of this invention are copper complexes in which coordination bonds are formed between the copper cation and the alpha-amino group of the acid, in addition to the electrostatic attraction between the cation and the carboxyl ions. This complex salt involving both coordination bonds and electrostatic attraction seems to enhance the bioavailability of the copper.

For 1:2 complexes, the formula illustrating both the coordination bond and the electrostatic attraction can be represented as follows :

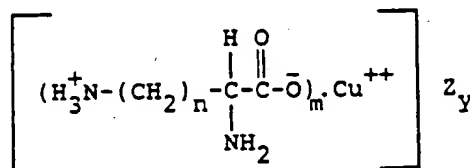


For 1:1 complexes, the formula illustrating both the coordination bond and the electrostatic attraction can be represented as follows:



wherein "y" is a number required to electrostatically balance the salt and "Z" is an anion.

While the above formulas represent the compounds sterically, the compounds can be represented as well by the following formula:



wherein

"n" is 1-5, "m" is 1 or 2, "Z" is an anion, and "y" is the number required to electrostatically balance the salt. It is important to remember that these salts are capable of existing in stable solid state form. They are thus processible and useable in animal feeds. This is to be distinguished from mere existence in non-isolatable transient states in an equilibrium of a solution. Preferred salts are those of the alpha-amino acid lysine wherein "n" equals 4. The most preferred salt is copper lysine sulfate either the 1:2 or the 1:1 complexes. In the formula presented, "Z" equals the anion, and may be an inorganic anion or an organic anion. Preferably, "Z" is selected from the group consisting of chloride, bromide, iodide, sulfates and phosphates. By the term "sulfates" and "phosphates" it is intended to include acid sulfates and acid phosphates, as well as those having no hydrogen moieties associated with either the sulfate or the phosphate, i.e., simply the sulfate  $\text{SO}_4^{--}$  and the phosphate  $\text{PO}_3^{-3}$ .

For the preferred solid form copper complexes of lysine and copper chloride, or copper sulfate, the complexes are stable, very water soluble, blue crystalline materials.

The 1:1 complexes are slightly less stable but are also more economical because they consume less lysine in manufacture. Generally, 1:1 complexes will be achieved if the pH is controlled on the more acid side, for example, from about 3.0 to about 4.0. It is believed that even the more stable 1:2 complexes are converted to 1:1 complexes in the acid environment of the gut.

For details of other copper complexes of the hydroxy acids for similar uses, see co-pending and commonly assigned application for COPPER COMPLEXES OF ALPHA HYDROXY ORGANIC ACIDS AND THEIR USE AS NUTRITIONAL SUPPLEMENTS, Serial No. 260,023, filed October 20, 1988.

Simple and economically feasible processes of preparing these copper complexes have been developed. In accord with the process of this invention, a solution of the lysine monohydrate is heated to 60-80°C. Hydrochloric acid is carefully added followed by the careful addition of copper oxide. The mixture is stirred for approximately 30 minutes. The small amount of insoluble copper oxide is removed by filtration. The filtrate is concentrated to dryness using conventional methods, such as hot air oven, spray drying, freeze drying or evaporation under reduced pressure, to give the desired product.

The same products may be obtained by carefully mixing a solution of lysine monohydrochloride with basic cupric carbonate. The mixture is heated under reflux for two hours. The solution was filtered while hot and cooled to 20°C. Acetone is added step-wise to the cooled filtrate until turbidity is observed. The mixture is stored overnight at 4°C to precipitate the desired product. The desired product may also be obtained from the filtrate by using a conventional drying method. The desired product could be further purified by recrystallization from acetone-water.

Another convenient method for obtaining the copper complexes involves the careful addition of powdered copper sulfate to a hot solution of lysine monohydrate. The desired product is obtained from the mixture as described in the previous examples.

The formation of copper complexes of basic alpha-amino acids such as lysine, Ornithine and gamma amino butyric acid are commonly used as a means of protecting or blocking the alpha-amino group in synthetic reactions in which it is desired to derivatize the side chain amino group. (Ref. A.C. Kurtz, J. Biol. Chem., 180, 1253 (1949); R. Ledger and F.H.C. Stewart, R.J. Stedman and Vincent Du Vigneaud, J. American Chemical Society, 78, 5883 (1956).) However, the procedures used in the reactions involve small amounts of reactants and complex methods which are not applicable for the preparation of large amounts of product. Additionally, the copper complexes are not usually isolated in crystalline form and are not characterized.

A distinct advantage of the compounds of the present invention is that they are crystalline, stable and water soluble compounds which can be easily obtained in large quantities for use as feed additives at a relatively low cost. The compounds of this invention provide a readily available source of copper and the essential alpha-amino acid lysine.

The level of addition of the copper alpha-amino acids containing a terminal amino moiety of this invention for use as a feed supplement can vary over a wide range. Preferably, the level of addition is such to provide dietary intake of copper from about 0.2 parts per million to about 2.0 parts per million, and most preferably from about 1.0 parts per million. These levels are satisfactorily achieved when the amount of the preferred complex salt of copper lysine, including both the 1:1 and 1:2 complexes as well as others added to the animal feed, is from about 0.1 g per head of cattle per day to about 1.0 g per head of cattle per day, preferably 0.6 g per head of cattle per day. It should, however, be understood that other levels of addition can be utilized and that the precise level of addition is not, in fact, critical, it being adjusted for the conditions of the animals being treated with the nutritional supplement.

Importantly, one of the distinct advantages of the compounds of the present invention is that they are crystalline water soluble compounds easily processible and size reducible to a powder for use in a convenient feed supplement form. In other words, their physical form is one which is easily mixable with typical ruminant animal feeds.

In making nutritional supplements for addition to the diets of animals, it is preferred that the complexes of the present invention be added to carrier or filler materials for processibility, ease of handling and sale. Examples of suitable carriers include distillers fermentation solubles, feed grains, animal, poultry and fish by-products and meal, whey and other cellulosic carrier materials well-known in the trace mineral product preparation techniques of the art.

The following example is offered to illustrate, but not limit, the preparation of the compounds of the present invention.

#### EXAMPLE 1

Basic cupric carbonate (12 gm.) was added portionwise very carefully to a solution of lysine monohydrochloride (10 gm.) in water (100 ml.). The mixture was heated under reflux for 2 hours. The solution was filtered while hot. The filtrate was cooled to 20°C and acetone was added stepwise until turbidity was observed. The mixture was stored at 4°C overnight. The precipitate was collected and dried. An analytical sample was prepared by recrystallization from water-acetone. The sample was dried over phosphorus

pentoxide. The infrared spectrum of the product was recorded in KBr pellet and found to be compatible with the structure herein given. Elemental analysis for  $C_{12}H_{28}O_4N_4Cl_2Cu \cdot 2H_2O$  calculated found: C, 31.14 (13.51); H, 6.96 (6.93); N, 12.10 (11.86); Cl, 15.31 (15.15). Copper analysis using ashing procedure. Ash 14.33 percent; copper calculated at 11.44 percent from ash. Theory was 13.73 percent. This elemental analysis confirms the structure of 1:2 complexes.

#### EXAMPLE 2

Lysine monohydrate (16.7 gm.) was mixed with water (25 ml.) and the mixture was heated with stirring at 60-80 °C until all the solid was dissolved. Concentrated hydrochloric acid (9.8 ml. of 37.3 percent) was added very carefully. The stirring of the solution was continued during the addition of acid. Copper oxide (4.0 gm.) was added to the stirred solution and stirring was continued at 70 °C for 30 minutes. The hot mixture was filtered to remove unreacted copper oxide. The filtrate was concentrated to dryness to give the desired product (21 gm.). The product is very soluble in water and gives a clear deep blue solution. The color of the solid product depends on the degree of hydration. An anhydrous product has a yellow-green color, and as the solid becomes hydrated, it changes to deep blue. The solid has the same chemical and physical properties as that of the product obtained in Example 1.

#### EXAMPLE 3

Lysine monohydrate (800 pounds; 362.88 kg) was mixed with 1,400 pounds (635 kg) of hot water. The solution was mixed until all the lysine had dissolved (approximately 10 minutes). Next, stirring was continued while copper sulfate pentahydrate (600 pounds ; 272.16 kg) was slowly added.

After all the copper sulfate pentahydrate had been added to the lysine solution, stirring was continued and the solution was maintained at 160-180 °F (71-82.2 °C) for 10-15 minutes to allow the complexing reaction to occur.

The copper lysine complex was dried using a conventional spray dry technique. The resulting desired product was a green fine powder.

This compound, if fed to livestock, particularly swine, cattle and poultry and also for example, sheep, at the levels specified herein, will show that the copper is in a highly bioavailable form and will provide it at levels sufficient for adequate dietary intake of copper to provide normal healthy growth, good red blood cell production, proper pigmentation for hair and wool, and at sufficient levels for normal reproductive patterns.

#### EXAMPLE 4

This example prepares the 1:1 copper lysine complex. For a 1:1 complex, the ratio of copper sulfate pentahydrate to lysine hydrochloride should be 1.4:1.

Lysine monohydrochloride (365 pounds; 165.56 kg) was mixed with 1200 pounds (544.3 kg) of hot water. The solution was mixed until all the lysine had dissolved (approximately 10 minutes). Next, stirring continued while copper sulfate pentahydrate (500 pounds ; 226.8 kg) was slowly added.

After all the copper sulfate pentahydrate had been added to the lysine solution, stirring was continued and the solution was maintained at 160-180 °F (71-82.2 °C) for 10-15 minutes to allow the complexing reaction to occur.

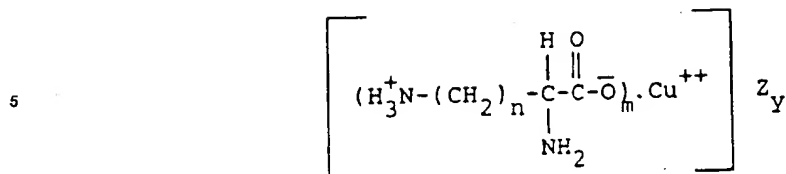
The copper lysine solution was dried using a conventional spray dry technique. The resulting desired product was a fine powder.

It therefore can be seen that the invention does and will accomplish all of its stated objectives.

#### Claims

1. A method of supplementing an animal's feed with bioavailable copper, characterized in that it comprises adding to said animal's feed an effective amount of a copper complex salt, in solid form, of the formula :



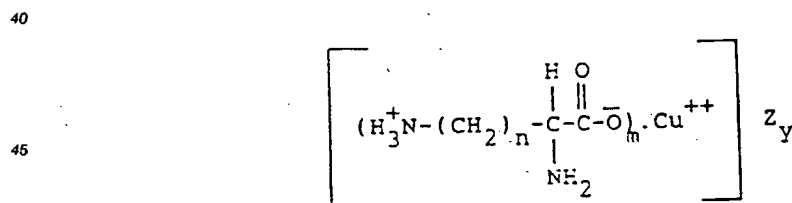


wherein "n" is from 1 to 5, "m" is 1 or 2, "Z" is an anion and "y" is the number required to electrostatically balance the salt.

2. The method of claim 1, wherein in the formula of said copper complex salt,  $n = 1$ .
3. The method of claim 1 or 2, wherein in the formula of said copper complex salt, "Z" is an inorganic anion.
4. The method of claim 3, wherein "Z" is selected from the group consisting of chloride, bromide, iodide, sulfates and phosphates.
5. The method according to claim 1, wherein said copper complex salt is the 1:1 copper lysine sulfate in solid form.
6. The method according to claim 1, wherein said copper complex salt is the 1:2 copper lysine sulfate in solid form.
7. An animal's feed, characterized in that it comprises, as an additive, a copper complex salt as defined in any one of claims 1-6.
8. Use of a copper complex salt, in solid form, as defined in any one of claims 1-6, for the manufacture of a feed additive in animal nutrition.

#### Patentansprüche

1. Methode, um Futter bioverfügbares Kupfer zuzufügen, dadurch gekennzeichnet, daß sie die Zugabe dem Futter einer wirksamen Menge eines Kupferkomplexsalzes in fester Form mit folgender Formel umfaßt:



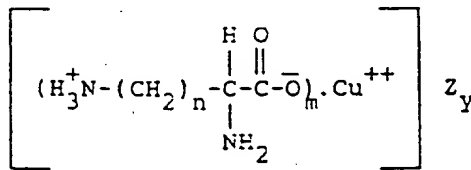
worin "n" 1 bis 5, "m" 1 oder 2 beträgt, Z ein Anion ist und "y" die zum elektrostatischen Abgleich des Salzes notwendige Zahl.

2. Methode nach Anspruch 1, bei der in der Formel des Kupferkomplexsalzes  $n = 1$ .
3. Methode nach Anspruch 1 oder 2, bei der in der Formel des Kupferkomplexsalzes "Z" ein anorganisches Anion ist.

4. Methode nach Anspruch 3, bei der "Z" innerhalb der Gruppe aus Chlorid, Bromid, Iodid, Sulfaten und Phosphaten ausgewählt wird.
5. Methode nach Anspruch 1, bei der das Kupferkomplexsalz das 1:1 Kupferlysinsulfat in fester Form ist.
6. Methode nach Anspruch 1, bei der das Kupferkomplexsalz das 1:2 Kupferlysinsulfat in fester Form ist.
7. Futter dadurch gekennzeichnet, daß es als Zusatz ein Kupferkomplexsalz nach einem der Ansprüche 1 bis 6 enthält.
8. Verwendung eines Kupferkomplexsalzes in fester Form nach einem der Ansprüche 1 bis 6 zur Herstellung eines Futterzusatzes.

#### Revendications

1. Procédé pour compléter un aliment pour animaux avec du cuivre biodisponible, caractérisé en ce qu'il comprend l'addition audit aliment pour animaux d'une quantité efficace d'un sel de complexe de cuivre, sous forme solide, de formule :



- dans laquelle, "n" est une valeur allant de 1 à 5, "m" est égal à 1 ou 2, "Z" est un anion et "y" est le nombre requis pour réaliser l'équilibre électrostatique du sel.
2. Procédé selon la revendication 1, suivant lequel, dans la formule dudit sel de complexe de cuivre, n = 1.
3. Procédé selon la revendication 1 ou 2, suivant lequel, dans la formule dudit sel de complexe de cuivre, "Z" est un anion inorganique.
4. Procédé selon la revendication 3, dans lequel "Z" est choisi dans le groupe constitué par le chlorure, le bromure, l'iodure, les sulfates et les phosphates.
5. Procédé selon la revendication 1, suivant lequel ledit sel de complexe de cuivre est le sulfate de cuivre lysine 1:1 sous forme solide.
6. Procédé selon la revendication 1, suivant lequel ledit sel de complexe de cuivre est le sulfate de cuivre lysine 1:2 sous forme solide.
7. Aliment pour animaux, caractérisé en ce qu'il comprend, en tant qu'additif, un sel de complexe de cuivre tel que défini dans l'une quelconque des revendications 1 à 6.
8. Utilisation d'un sel de complexe de cuivre, sous forme solide, tel que défini dans l'une quelconque des revendications 1 à 6, pour la fabrication d'un additif alimentaire en nutrition animale.